Cardiotoxic Activity of the Rhizome of Polygonatum sibiricum in Rats

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The cardiotoxic effect of the rhizome of Polygonatum sibiricum was investigated in the left atria of rats. The methanol extract of the rhizome of Polygonatum sibiricum (OM) (1—7 mg/ml) concentration-dependently increased the developed tension of the left atrium. It also strongly inhibited cAMP phosphodiesterase. The increase in cAMP level correlated the increase in left atrial contraction. On the other hand, OM did not inhibit Na⁺, K⁺-ATPase. The cardiotoxic effect of OM was strongly inhibited by reserpine, a sympatholytic agent. Furthermore, OM-treated left atria inhibited the tension produced by propranolol, a beta adrenoceptor antagonist. These findings suggested that the cardiotoxic effect is due to stimulating beta adrenoceptors through activation of sympathetic nerves.

Key words Polygonati Rhizoma; cardiotoxic effect; beta adrenoceptor

MATERIALS AND METHODS

Materials Polygonati Rhizoma collected in China by Tochimoto Tenkaido Co., Osaka (Japan). Crushed rhizome (4 kg) was successively extracted with 101 methanol on a heating bath. The methanol extracts (OM, 183 g) were lyophilized and stored at 4°C until just before use. A voucher specimen was deposited at the herbarium of Toyama Medical and Pharmaceutical University.

Animals Adult male SD rats (body weights 150—200 g) were used for all studies. They were housed individually in an air-conditioned room at an ambient temperature of 24±1°C with a 12 h light-dark cycle. The animals were kept in the experimental animal room for 7 d with free access to food and water.

Effect of OM on Tension Developed in the Left Atria of Rats

The left atrium was isolated rapidly under ether anesthesia and was placed in a 20 ml bath containing Krebs-Henseleit solution (118 mM NaCl, 4.7 mM KCl, 2.55 mM CaCl₂, 1.18 mM MgSO₄, 1.18 mM KH₂PO₄, 24.88 mM NaHCO₃, 1.11 mM glucose, 37°C, 95% O₂—5% CO₂) under a weight of 1 g. After 1 h, On electrical stimulus (SEN-3301, Nihonkoden, Japan) was performed directly the muscle (frequency: 1 Hz, duration: 5 ms, increment 15% of maximum voltage controlled by isolator, SS-102J, Nihonkoden). The developed tension was measured by a strain-gauge tension meter (TB-612T, Nihonkoden), and then recorded with a pen-recorder (WT-645G, Nihonkoden). The test samples were dissolved in 0.9% saline or dimethyl sulfoxide (DMSO) solution (ex-

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Fig. 1. Effect of Ousel Methanol Extract (OM) on Tension Developed in the Rat Left Atrium
Each value represents the mean ± S.E. from 4—6 rats.

Table 1. Effect of OM on Na\(^+\)-K\(^+\) ATPase Activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>N</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OM</td>
<td>1 (mg/ml)</td>
<td>4</td>
<td>3.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7</td>
<td>8.5 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6</td>
<td>18.8 ± 2.6</td>
</tr>
<tr>
<td>Ouabain</td>
<td>0.1 (μM)</td>
<td>7</td>
<td>1.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6</td>
<td>35.4 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6</td>
<td>80.9 ± 0.9</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. N: number of experiments.

Table 2. Effect of OM on cAMP-PDE Activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>N</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OM</td>
<td>20 (μg/ml)</td>
<td>5</td>
<td>9.9 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>5</td>
<td>19.7 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>6</td>
<td>25.5 ± 5.1</td>
</tr>
<tr>
<td>Papaverine HCl</td>
<td>25 (μM)</td>
<td>4</td>
<td>35.3 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>4</td>
<td>52.1 ± 1.7</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. N: number of experiments.

Effect of OM on cAMP-PDE Activity
OM (100 μg/ml; maximum concentration) inhibited 25.5 ± 5.1% of the cAMP-PDE activity (Table 2). The inhibition of OM was concentration-dependent. Papaverine hydrochloride, a positive control, strongly inhibited this activity.

Effect of OM on cAMP
The cAMP level in the left atrium is shown in Fig. 2. OM (7 mg/ml) was two-fold greater than the control. The increase cAMP level correlated with the increase in left atrial contraction. Epinephrine increased the cAMP concentration-dependently.

Effect of OM on the Sympathetic Nervous System
The effect of OM on the sympatholytic left atrium is shown in Fig. 3. The cardiotonic effect of OM was strongly inhibited by treatment with reserpine, a sympatholytic agent. In the basic study, we examined the sympatholitic effect in terms of its concentration-dependence after treatment with tyramine hydrochloride, and found that tyramine hydrochloride had an effect at 0.3 and 3 μM (data not shown).

The effect of OM on an alpha-adrenergocceptor antagonist is shown in Fig. 4. No change in the increment of contractile force by OM was observed between the phentolamine (10 μM) (alpha-adrenergocceptor antagonist) treated and an untreated left atrium. In the basic study, we examined the effect of an alpha-adrenergocceptor in terms of its concentration-dependence after treatment with phenylephrine, and found that phenylephrine had an inhibitory effect at 10 μM, and the inhibitory effect was abolished by phenylephrine (1, 10 and 100 μM), an alpha-adrenergocceptor agonist (data not shown).

The effect of OM on a beta-adrenergocceptor antagonist
(propranolol) is shown in Fig. 5. The OM-treated left atrium inhibited the increase in tension developed. (In the basic study, we examined the effect of a beta-adrenoceptor in terms of its concentration-dependence after treatment with propranolol, and found that propranolol had an inhibitory effect at 4 μM, and the inhibitory effect was abolished by isoproterenol (0.05, 0.1 and 1 μM), a beta-adrenoceptor agonist (data not shown).

DISCUSSION

The present study clearly shows that OM produces consistent cardiotonic activity in the rat left atrium. The cardiotonic activity was observed with increasing cAMP. In addition, OM inhibited phosphodiesterase activity. These findings indicate that the cardiotonic activity of OM was due to increase sympathetic nervous system produced by rising cAMP. Moreover, the propranolol (a beta adrenoceptor antagonist) treated left atrium exhibited a cardiotonic activity between the control and OM group. However, the phenetermine (an alpha adrenoceptor antagonist) treated left atrium was not changed. From these findings, it seems likely that the cardiotonic mechanism of OM is due to stimulation of beta adrenoceptors postsynaptically. The above experimental results suggest that the cardiotonic activity of OM supports its traditional medical use.

REFERENCES